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RATIO OF PHOSPHATE, NITRATE, AND POTASSIUM ON ABSORPTION AND GROWTH[1]

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(WITH NINE FIGURES)

In this paper are reported the results of experiments obtained in connection with a study of the effects of harmful soil constituents upon plant growth and upon soil solutions and fertilizer action. The results here reported are especially with reference to the growth made by wheat seedlings in culture solutions containing many different ratios of phosphate, nitrate, and potash, and in regard to the ratio of these constituents originally present and removed by the wheat seedlings in the course of the experiment. In these investigations solution cultures containing the three fertilizer ingredients, namely P_2O_5, NH_3, and K_2O, as calcium acid phosphate, sodium nitrate, and potassium sulfate, respectively, in all possible ratios of one, two, and three constituents, varying them in stages of 10 per cent, were prepared, the concentration being 80 parts per million in these constituents. The selection of the salts as carriers of phosphate, nitrate, and potash, and the statement of the results in terms of P_2O_5, NH_3, and K_2O, are in harmony with the practice in fertilizer work and, for the sake of simplicity, these designations are also retained in the present paper. The salts selected, it will be seen, are also carriers of calcium, of sodium, and of sulfate, and the three salts, therefore, are the best that could be selected for giving at the same time other needed constituents.

The culture solutions of these three salts contained in each case a total concentration of 80 parts per million of P_2O_5, NH_3, and K_2O,

[1] Published by permission of the Secretary of Agriculture.

but these varied, as above noted, in the ratio in which they were present. Wheat plants were grown in these various cultures and observations were made in regard to general development, the effect on root growth, and appearance. The green weight of the plants was taken at the termination of the experiment. The solutions were changed every three days and an analysis made, the phosphate, nitrate, and potash being determined, thus giving the concentration of these constituents and the ratio existing at the end of every three-day period for comparison with the original concentration and ratio. This changing of the solutions was kept up for twenty-four days, thus making eight changes. In this work the methods[2] devised in these laboratories for the determination of small amounts of such constituents rendered excellent service and point a way for their further use upon other problems in connection with the biochemical relationships of soils and plants, which have hitherto been impossible of attack. In the discussion and presentation of the results, the triangular diagram as used in physical chemistry was employed, and has proven very useful as a guide in the work for the systematic handling of the experimental details. The results can best be presented and interpreted by its means, and the method should prove very useful as a guide in other lines of experimental work where similar relationships are involved.

The use of the triangular diagram as a guide in the work

The number of solution cultures required in order to have all the possible ratios as outlined above is sixty-six. To bear in mind these ratios for three different ingredients, together with the ratios of the solutions after the plants had grown, and perhaps also the ratios of the material removed from the solution, a total of 594 numbers, is practically an impossibility, and it is readily seen that in order to discuss such a comprehensive experiment as this the material must be reduced to a workable basis, so that the various phases of the results can be kept in mind and the proper correlation and comparisons made. The triangular diagram as suggested by SCHREINE-

[2] SCHREINER, O., and FAILYER, G. H., Colorimetric, turbidity, and titration methods used in soil investigations. Bulletin 31, Bureau of Soils, U.S. Dept. Agric. 1906.

MACHER[3] in 1893 and again by BANCROFT[4] in 1902 has been of the greatest service to physical chemistry, where both theoretical and practical consideration of percentage composition of three component parts are concerned.

In the present investigation we have likewise to consider the three component parts of the fertilizer mixture; namely P_2O_5, NH_3, and

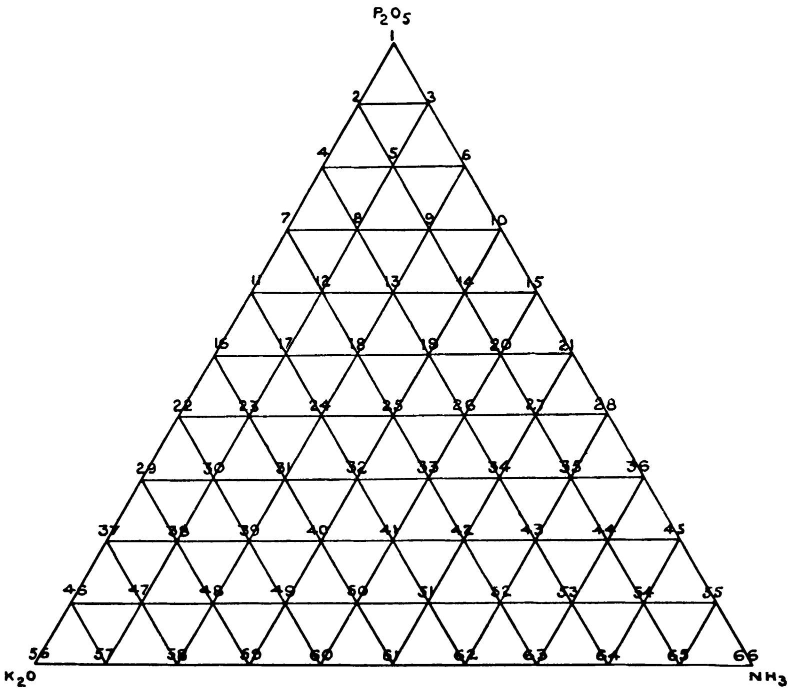

FIG. 1.—Showing the triangular diagram, with the points numbered, which represent the 66 culture solutions.

K_2O. It is possible, therefore, to represent any mixture of these three component parts in the triangular diagram. Such a triangular diagram is shown in fig. 1. It is an equilateral triangle in which the extreme points of the angles represent 100 per cent respectively of

[3] SCHREINEMACHER, F. A. H., Konzentrierung oder Verdünnung einer Lösung bei Konstanter Temperatur. Zeit. Phys. Chem. 11:81. 1893.

[4] BANCROFT, W. D., Synthetic analysis of solid phases. Jour. Phys. Chem. 6:178. 1902.

each of the ingredients, P_2O_5, NH_3, and K_2O, as shown in the diagram. Each side of the triangle is divided into ten equal parts, and lines are drawn connecting these points.[5] In the diagram, for the sake of ready reference, the intersections of these lines have been numbered. If we consider the line representing the base of the triangle, it is obvious that the point which represents 100 per cent K_2O (number 56 in the diagram) represents at the same time 0 per cent NH_3, and the point 100 per cent NH_3 (number 66) likewise represents 0 per cent K_2O. If we take a point half-way between these two points, for instance point 61, we have a mixture of the two salts in equal proportions; i.e., the fertilizer constituents represented by that point will be 50 per cent K_2O and 50 per cent NH_3. Similarly, point 16 represents 50 per cent K_2O and 50 per cent P_2O_5, and point 21 represents 50 per cent NH_3 and 50 per cent P_2O_5. If we take a point nearer to either of the corners, we will have a higher percentage of that ingredient and a correspondingly lower percentage of the other. For instance, at the point 59 the composition is 70 per cent K_2O and 30 per cent NH_3; at 29 it is likewise 70 per cent K_2O, but 30 per cent P_2O_5; at 64 it is 20 per cent K_2O and 80 per cent NH_3; at 45 it is likewise 80 per cent NH_3, but 20 per cent P_2O_5.

As stated above, points on the base line 56 to 66 represent mixtures containing no P_2O_5. The next line above this, namely 46 to 55, represents mixtures containing throughout 10 per cent P_2O_5, but varying amounts of the other two constituents. Similarly, the line 37 to 45 represents throughout 20 per cent mixtures of P_2O_5; line 29 to 36, 30 per cent mixtures of P_2O_5; and so on upward until point 1, the apex of the triangle, is reached, where the composition is 100 per cent P_2O_5, as already explained. Similarly, points on the line 1 to 66 represent 0 per cent K_2O; line 2 to 65 represents 10 per cent K_2O, but varying amounts of P_2O_5 and NH_3; and so on until at point 56 the composition is 100 per cent K_2O. Likewise, points on the line 1 to 56 represent 0 per cent NH_3; line 3 to 57 represents 10 per cent NH_3, but varying amounts of P_2O_5 and K_2O; and so on until at point 66 the composition is 100 per cent NH_3. It is obvious,

[5] Such diagrams for physical chemical work, giving still finer rulings, namely 100 to each line, can be purchased from the Cornell Co-operative Society, and were used in these investigations.

therefore, that any point within the triangle represents a mixture composed of the three constituents, its position in the triangle being determined by the composition of the mixture, namely the ratio of the three component parts, P_2O_5, NH_3, and K_2O. For instance, point 12, being on the 60 per cent phosphate line, represents this composition of P_2O_5, namely 60 per cent, and being at the same time on the 10 per cent NH_3 line, and the 30 per cent K_2O line, it represents 10 per cent and 30 per cent of these constituents, respectively. The composition of the mixture represented by this point is, therefore, P_2O_5 60 per cent, NH_3 10 per cent, K_2O 30 per cent; i.e., the ratio of these constituents in the fertilizer mixture is 60–10–30 or 6–1–3. Similarly, the point 34 represents the following mixture of the composition: P_2O_5 30 per cent, NH_3 50 per cent, K_2O 20 per cent, or a fertilizer ratio of 3–5–2.

For the sake of convenience in stating such ratios or percentage composition of the fertilizer mixtures in this investigation, the figures are always given in the order P_2O_5, NH_3, and K_2O, as shown above.

The triangle, therefore, represents single fertilizer constituents at the apices or vertices, mixtures of any two constituents along the boundary lines of the triangle, and mixtures of all three constituents within the triangle.

Solution culture methods

Before proceeding with a description of the general appearance of the cultures growing in solutions with the different fertilizer ratios, it may be well to describe briefly here the solution-culture method used in these experiments, as the manner of procuring a sufficiently large number of seedlings and of preparing physiologically pure water are important factors in carrying on this or allied investigations.

METHOD OF PROCURING UNIFORM SEEDLINGS

In the work under consideration, as well as in other work in progress in these laboratories, it is necessary to have a large number, often several hundred and sometimes thousands, of uniform seedlings; i.e., seedlings of the same age and equal in development and general vitality. The general principle on which this equal germination of

wheat seedlings is based has already been described by LIVINGSTON[6] and in former bulletins of this bureau.[7] The method consists in having a perforated disk, supported by ordinary corks in such a way that it will just float upon the surface of a pan of water. In the earlier work a wire gauze, which had been coated with paraffin so as to make virtually a plate of paraffin reinforced by wire, was used, holes being made in this plate by means of a hot wire. Perforated cork sheets, preferably paraffined, have also been used. In this case sheet cork about one-eighth of an inch in thickness is immersed in melted paraffin, and after removal from the paraffin this is allowed to harden in the air spaces of the cork. Holes are then made by means of a small cork borer so as to give a perforated plate. Both of these plates are open to objections. In the paraffined plate, when it is used continually, there is a tendency for the paraffin to split off from the wire, which is thus exposed to the action of the water and the roots; moreover, this plate is not easily repaired. The cork plate, on the other hand, shows considerable tendency to warp, is rather fragile, and is not easily kept sterile.

Instead of these plates there were used in these present experiments perforated hard-rubber sheets, thus overcoming the above objections to a considerable degree. These are prepared by cutting a circular disk of 305 mm. from vulcanized sheet rubber 3.2 mm. in thickness. By clamping several of these tightly together, preferably between layers of wood, small holes are drilled through the mass approximately 4.8 mm. in diameter and 5.0 mm. apart. Disks of this material float level upon the water when supported by corks, are not so subject to warping, and are readily cleaned and kept sterile. The corks are fastened to the under surface of the disk in four or five places on the circumference and in the center. For this purpose either rubber or wooden pegs or wire may be used; if the latter, it should be made either of iron or aluminum, never of brass or copper. The size of the corks is so gauged by trial that the disk is just supported on the surface

6 LIVINGSTON, B. E., A simple method for experimenting with water cultures. Plant World **9**:13. 1906.

7 SCHREINER, O., and REED, H. S., Some factors influencing soil fertility. Bulletin 40, Bureau of Soils, U.S. Dept. Agric. 1907.

of the water. After floating this perforated disk on the surface of the water, the wheat seed, which has been previously soaked for about two hours, not longer, is spread evenly over the surface. This method insures an even germination of the seed and has the advantage of keeping the seed and later the young seedlings just moist. The seeds are never submerged in the water, nor do they remain suspended dry above the water. The roots grow through the holes of the disk into the liquid below. This method of sprouting wheat seedlings is far superior to growing them in sand, since the seedlings are more uniform and the apparatus can be kept sterile much more readily than sand. Furthermore, for water culture purposes the seedling is readily removed by merely lifting it from the disk. In this way the roots or delicate root hairs suffer no injury whatever, while in removing seedlings from sand some injury would be unavoidable. Moreover, the seedlings are removed direct from water to grow in solutions, and thus have the advantage of germinating in a medium similar to that in which they are to be grown.

The water in the germinating pans may be distilled water, if desired, or where good tap water can be secured this may be used. The water in the pans is changed daily, or, during the warm weather, twice a day. Several hundred uniform seedlings can be procured from a single disk of the kind described. Whatever inferior plants occur are rejected. For the bottle culture work described in this bulletin, the seedlings were used when the plumule was about 2 cm. high and just ready to emerge from the enveloping sheet.

More recently disks of perforated aluminum have been used in this laboratory and have proven very satisfactory. These disks are floated by means of a raft of sealed glass tubing of such dimensions as is required to float the plates in the manner above described. Three lengths of tubing 102 cm. long, 35 mm. in diameter, with approximately a 1.5 mm. wall thickness, were sufficient to float six aluminum disks 30 cm. in diameter and 1.6 mm. in thickness. The lengths of sealed tubing and two glass rods 56 cm. long and 12 mm. in diameter are wired together into a raft. The entire arrangement is floated in a porcelain-lined iron tank. Fig. 2 shows this tank with seedlings at various stages of development.

NOTCHING CORKS AND MOUNTING SEEDLINGS

The bottles used in these cultures are made of flint glass, have a capacity of about 250 cc., a total height of 100 mm., an outside diameter of 70 mm. at the bottom, a neck about 20 mm. high, and a mouth 57 mm. in diameter. This bottle is stoppered by means of a soft, flat cork about 12 mm. in thickness and notched for holding the seedlings. The method of notching these corks consists in cutting ten vertical, triangular wedges from the circumference. Each wedge after being cut out is truncated, so that when replaced a small triangular opening, through which the plumule of the seedling will pass,

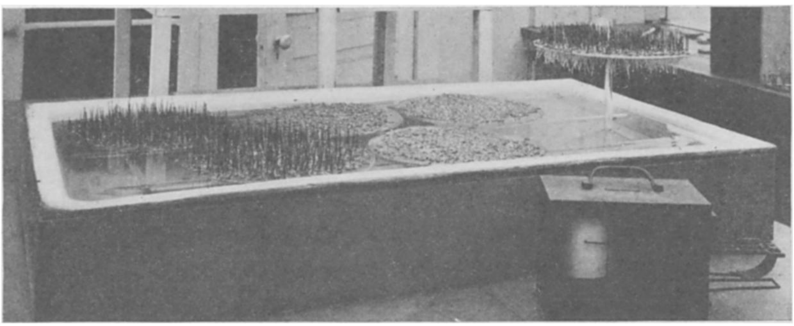

FIG. 2.—Method of germinating wheat seedlings on aluminum disks, which are floated in an enameled tank; disk to right elevated to show the plant roots.

is formed. This hole should be large enough to hold the seedling firmly and yet not bruise or injure it in any way by pressure. Around the circumference of the cork and in the upper half a groove is made sufficiently large to hold a small rubber band. After the wedges are inserted, the band keeps them in place and allows the cork with the seedlings to be handled readily and put into or taken out of the bottle without disturbing the plants. The seedlings are most easily inserted in the cork in the following manner: The cork with the ten wedges held in place by the rubber band is taken in the left hand and the seedling in the right hand. The plumule is pushed through the small, triangular opening, and then pulled up until the seed is close against the cork. When older seedlings, in which the leaves have unfolded

themselves, are to be used for an experiment, or where plants such as cowpeas are used, the wedge must be removed, the seedling put in place, and the wedge and rubber band replaced.

PREPARATION OF PHYSIOLOGICALLY PURE WATER

It is safe to say that ordinary distilled water, such as is commonly found in laboratories, is unsuited for culture experiments. This is due to a variety of causes which need not be entered into here, but which are discussed in a bulletin by LIVINGSTON.[8] Such water, while pure from a chemical and physical standpoint, is nevertheless not suited for such delicate indicators as plants, which are highly susceptible even to very minute quantities of toxic materials. Such water can be improved by distillation with strong oxidizing agents, such as potassium permanganate in alkaline solution or potassium bichromate in acid solution. The far simpler method already described by LIVINGSTON[8] has given very satisfactory results in this laboratory. In this method the water is purified by shaking it with a highly absorptive carbon black, which removes from the water any traces of injurious bodies it may contain. Not all carbon black possesses this property to an equal degree; the variety used in this laboratory is known as the "G Elf" brand, and is prepared on a commercial scale by burning natural gas and condensing the finely divided carbon on cooled surfaces. It is the same carbon black used for the purpose of decolorizing soil and plant solutions in this laboratory. For the present purpose the carbon black is thoroughly washed with distilled water as an added precaution, although it is not definitely known whether anything is thereby removed from it or not. The carbon black is then kept in this moist condition mixed with water so as to form a thin paste. About 10 cc. of this paste is added to each liter of water which is to be purified, and after shaking the mixture is allowed to stand for fifteen to thirty minutes and then filtered through an ordinary filter paper into a clean, hard-glass receptacle. Water treated thus is very satisfactory for the growth of plants in solution cultures.

[8] LIVINGSTON, B. E., Further studies on the properties of unproductive soils. Bulletin 36, Bureau of Soils, U.S. Dept. Agric. 1907.

General behavior of the cultures containing different fertilizer ratios

The plants in the different cultures prepared as described in the previous section show some marked differences almost from the very outset. After several changes of solution have been made, these differences become quite prominent. The most marked of these is

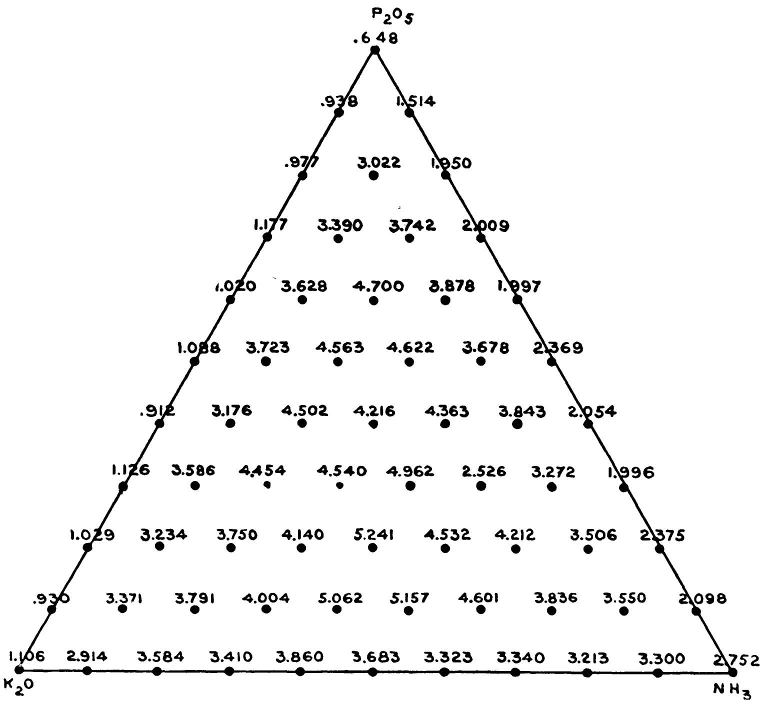

FIG. 3.—Green weight of 10 wheat plants grown in 66 cultures with different ratios of P_2O_5, NH_3, and K_2O.

that the solutions containing only one or two of the fertilizer ingredients, i.e., the entire periphery of the triangle, show a markedly less development of the plants than the cultures in the interior of the triangle wherein the three elements are combined. In other words, even the addition of only 10 per cent of the third element to mixtures of the other two produces a very appreciable increase in plant growth. This effect is most noticeable in the change from no nitrate to 10 per

cent nitrate. Of the single fertilizer ingredients, the nitrate culture is the best, and this is usually followed by the potassium culture, the phosphate culture being in general the poorest of the entire triangle. The cultures in the interior of the triangle do not show so marked a difference, although it is apparent that there is a tendency toward a rise in the middle of any line, this being displaced somewhat along some of the lines so as to give the effect of the highest and better region of growth lying in the middle of the lower part of the triangle; i.e., nearer to the potassium-nitrogen base. The set grew from February 25 to March 21. The green weights obtained in the sixty-six cultures are given in the triangular diagram shown in fig. 3. A more detailed discussion as well as grouping of these green weights, their correlation with the concentration of the solution, and the amount of nutrients removed will be given later.

As has already been stated, the solutions were analyzed every third day for the three component fertilizer parts, phosphate, nitrate, and potash, expressed as P_2O_5, NH_3, and K_2O. The original concentrations in these elements were in the sum total 80 parts per million. After the analysis the sum total of the three component parts was again calculated and the average concentrations of these three elements was again ascertained for the eight periods. The average concentrations will be found in the diagram in fig. 4. It is thus apparent that more of the essential ingredients were removed in the interior of the triangle; i.e., there was a greater absorption where all three were combined than where only two elements were present, and the least removal took place in those cultures where the single salts were present. It is also noticeable that in the solutions containing the three fertilizer elements the greatest removal occurred in that region of the diagram where the greatest growth occurred. A more detailed discussion of these results and their component parts will be given later in this paper.

Ratios of P_2O_5, NH_3, and K_2O, found in the various cultures

It might be said that in all cases the ratio in the final solution was never the same as the original ratio. The amount of change which had taken place, however, was markedly different in the different solutions and depended largely upon what the original ratio was. In

order to show this change in ratio of the fertilizer ingredients, the triangular diagram is of the greatest service, for without this it would be impossible to get an intelligent idea of what had occurred in the solution. In the diagram fig. 5 are given the original ratios of the fertilizer constituents, the ratios left in these solutions as shown by analysis, and the corresponding ratio of the removed constituents.

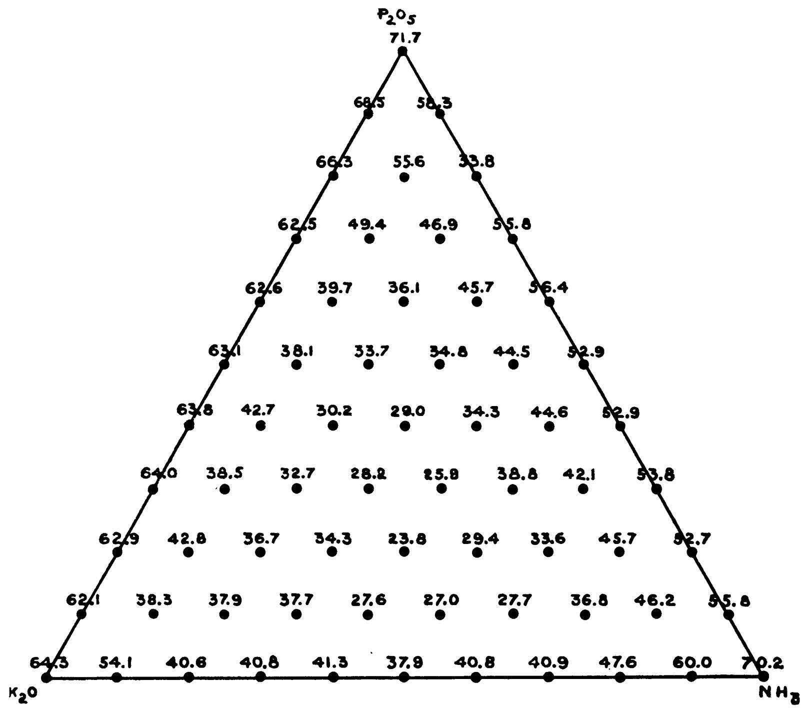

FIG. 4.—Showing the average concentration in parts per million of P_2O_5, NH_3, and K_2O of the solution after the growth of 10 wheat plants; concentration of the original solution was 80 ppm.

It may be shown mathematically that these three points lie upon a straight line and this is the case in the diagram.

The large dots in the diagram represent the original ratios corresponding to the scheme previously explained and given in fig. 1. The circles indicate the ratio left in the solution as shown by analysis. The other end of the line indicated by an arrow point shows the corresponding ratio of the removed materials. It must be borne in mind that

this diagram deals only with the ratios of the ingredients and not with the amounts that are present.

It is apparent that there is a decided tendency for these lines to converge toward a region somewhat below the center, as shown by the congregation of arrow points in this region. In other words, the solutions near this central area change least in their ratio, and the

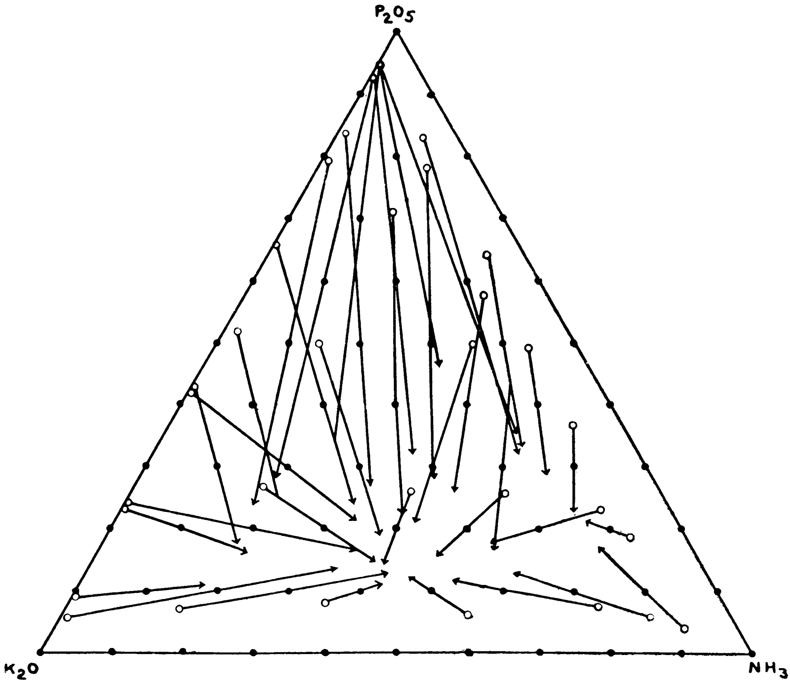

FIG. 5.—Showing the ratio of the original, the final, and the ratio of the loss of P_2O_5, NH_3, and K_2O from the culture solution: the dots indicate the ratio of the constituents in the original solution; the circles show the ratio of the constituents in the solution after growth; and the arrows show the ratio of the decrease.

farther the ratios were removed from this central area originally, the more were they altered in the course of the experiment. According to the diagram this area would seem to lie between the 10 and 20 per cent phosphate line, since the points on the lower line have moved in a direction opposite to that taken by the ratios in the upper part of the triangle. The data already presented show in general that the area of greatest growth occurred in this same region. It is in

this region of greatest growth, therefore, that the greater absorption took place with the least change in ratio; in other words, the solutions represented by this region offered the best environment for plant development and the best ratios for the absorption of plant nutrients.

Attention might also be called to the fact that in the ratios where the nitrate was low, there has been a movement to the no nitrate line, or at least so close to this line that it was impossible to plot them otherwise. After the comparatively small amount of nitrate was removed or reduced to a minimum, the point marking the ratio would have to move along the no nitrate base line in the direction either of the potash or of the phosphate, depending upon which was removed in the larger amount. The diagram shows that this movement of the ratio in the solutions was in nearly all cases toward the phosphate apex of the triangle. It is obvious that such a condition of affairs will cause a shifting of the lines connecting the ratios in the diagram, and that a similar state of affairs in the cultures on the low potassium line would produce a shifting of the ratio lines in the opposite direction. An examination of the diagram shows this has occurred, because the ratio lines in the upper part of the triangle show a divergence, giving a fanlike effect. In the case of the potash this divergence is also strongly noticeable, although the potash was never so completely removed as was the case with the nitrate.

The results of the experiments considered by periods

The very earliest periods, when compared with the later periods, show some differences in the ratio in which the three elements are removed, as has already been pointed out. Mention has been made of the fact that the ratios removed from the solutions along the lower phosphate line, namely, the 8 parts per million line of cultures, had a tendency to cause the ratio lines to turn from a point below this line to points above the line with increase in time. This general behavior of the cultures is very well shown by the three diagrams for the first, second, and third periods. The first diagram (fig. 6) shows the arrow points of the 10 per cent phosphate mixtures to lie below this line. The second diagram (fig. 7) shows some of the points above and some still below the line. In the third diagram (fig. 8) all but one arrow point lie above the line, and some arrow points of the 20 per cent

phosphate line are also noticed to have turned so far that some of them take a position even slightly above this line. There is a general tendency, moreover, for all of the points to lie somewhat higher for the other cultures, thus showing that this influence is probably common to all the cultures, though seen most strikingly in those containing small amounts, where an actual reversal takes place. This

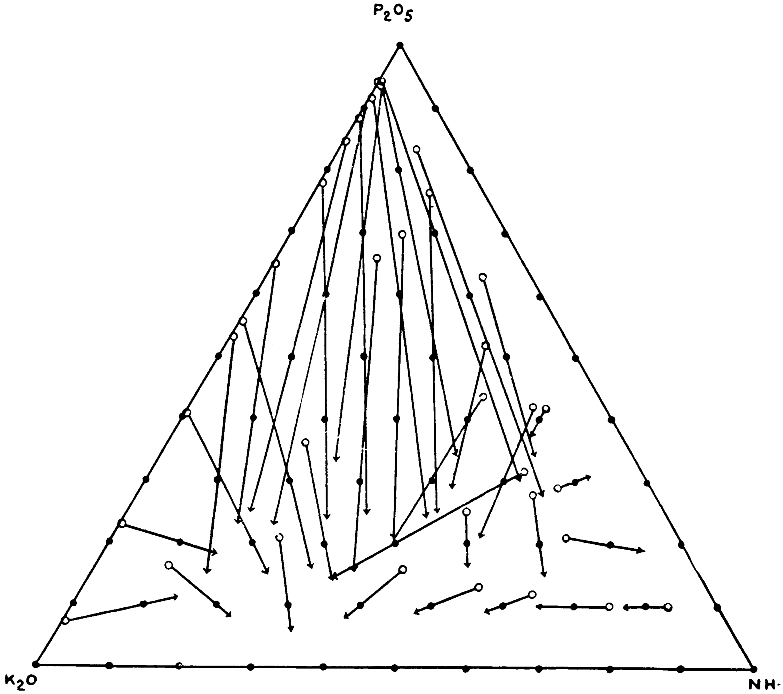

FIG. 6.—Showing the ratio of the original, the final, and the ratio of the loss of P_2O_5, NH_3, and K_2O from the culture solution in the first period.

general result may be interpreted to mean that the relative amount of phosphate removed in the early stages of the seedlings is not so great as it is subsequently, and the phenomenon can probably be correlated with the fact that in experiments where distilled water is used rather than these solution cultures, phosphate is excreted by the germinating seed and can be detected in the water in which germinating seeds or young seedlings bathe. Although the seedlings when used in these experiments have passed in the main through this

stage, the results show that there was a material lessening of the power to remove phosphate, probably due to the fact that the process of the phosphate absorption had not fully replaced the opposite function existing during germination and early growth.

The potassium absorption is also different from that in the later periods, although this is not so striking in the diagrams as in the case

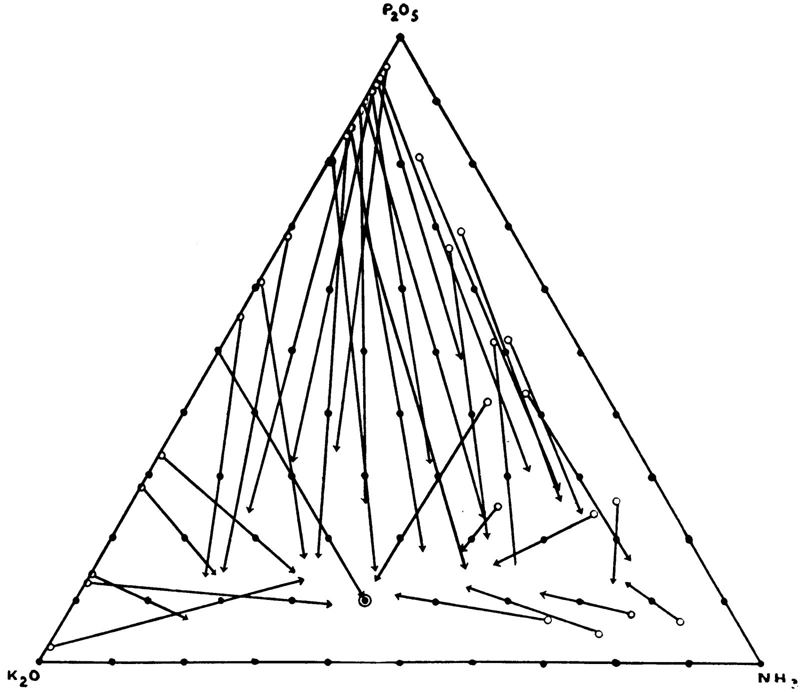

FIG. 7.—Showing the ratio of the original, the final, and the ratio of the loss of P_2O_5, NH_3, and K_2O from the culture solution of the second period.

of the phosphate. The average ratio of the removed material during these first three periods for all the cultures is 19 for phosphate, 39 for nitrate, and 42 for potassium; whereas the average for the five succeeding periods is 21 for phosphate, 44 for nitrate, and 35 for potash, thus showing that there is a tendency for a relatively greater potash and a relatively less phosphate absorption to take place during the earlier periods. So far as the influence of these fertilizer elements on growth is concerned, however, it is to be noticed that the nitrate has the

greatest effect, as is shown by the fact that the difference between the no nitrogen line and the 8 ppm. line is very great in the case of the nitrate, less in the case of the potash, and least in the case of the phosphate. As a rule, beyond the second or perhaps the third period the diagrammatic representation of the result is on the whole uniform, but is influenced undoubtedly by the conditions of growth during any

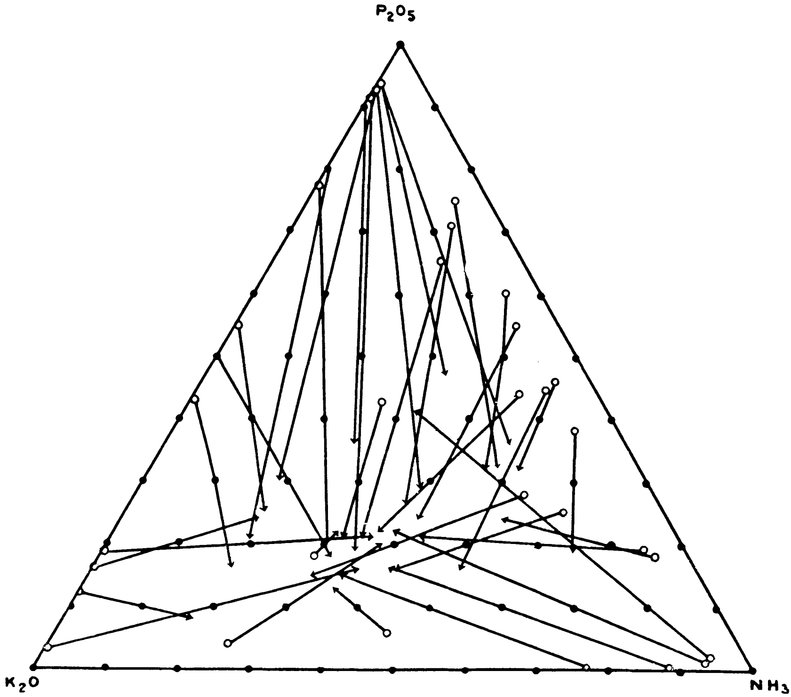

FIG. 8.—Showing the ratio of the original, the final, and the ratio of the loss of P_2O_5, NH_3, and K_2O from the culture solution in the third period.

period; in other words, by weather and other conditions, which is shown perhaps the quickest in the nitrate removal from the cultures.

Some remarks should here be made concerning the possible influence of bacteria. In the course of these experiments bacteria and other microorganisms were excluded so far as possible, but no special effort was made to maintain absolutely sterile conditions, inasmuch as this would have been a practical impossibility in an experiment on so large a scale. Moreover, it may even appear questionable whether

absolute sterility would be desirable. The bottles were sterilized before being used in making culture solutions for the various changes, the pans and other apparatus used in germinating the seed were sterilized from time to time, and corks used for the cultures were always clean and sterilized before use. Although all of these precautions were taken, it was of course not possible to exclude some microorganisms in such work, as the solutions were exposed from time to time to the air. There was in no case any excessive microorganic life noticeable. While bacteria and other microorganisms were present in the cultures to a slight and, under the conditions, unavoidable extent, it can hardly be said that their influence could have been large; that is, such influence as they had was probably so slight as to be negligible so far as the general and larger tendencies which are shown in this paper to exist are concerned.

Controls which were set up without having plants grown in them were found not to have changed their concentration or proportions of the constituents. Moreover, the various cultures support each other in their general tendencies. If the disappearance of nitrates were to be ascribed to bacterial activity, this should have shown itself all the more prominently as the age of the cultures increased. Such changes as were noticed from period to period might be ascribed to changes in the climatic conditions, thus still further affecting the plant's metabolism. This is very nicely illustrated in a series of preliminary experiments in which the solutions were changed every day instead of every three days, as was finally done. The diagram giving the results obtained on a clear day shows strikingly a relatively greater nitrogen removal under these conditions. The diagram giving the results obtained on the following day, which was cloudy and rainy, shows no less strikingly that relatively less nitrogen was absorbed on the cloudy and rainy day. It would be obviously unfair to conclude that under such conditions bacterial activities had been greater on the sunny day than on the following rainy day, especially as this result is in harmony with all observations on the removal of nitrate from solutions by plants. The processes of nitrogen utilization within the plant are known to be greater under conditions of better illumination. It is fair to assume, therefore, that this same general tendency held in all the other cultures, and that whatever

effect the bacteria may have had, it was slight in comparison to the activities of the plant itself under the conditions of this experiment.

Relation between growth and concentration of P_2O_5, NH_3, and K_2O found in the various cultures

The experimental results reported seem worthy of a closer analysis than that accorded them thus far. General tendencies as indicated

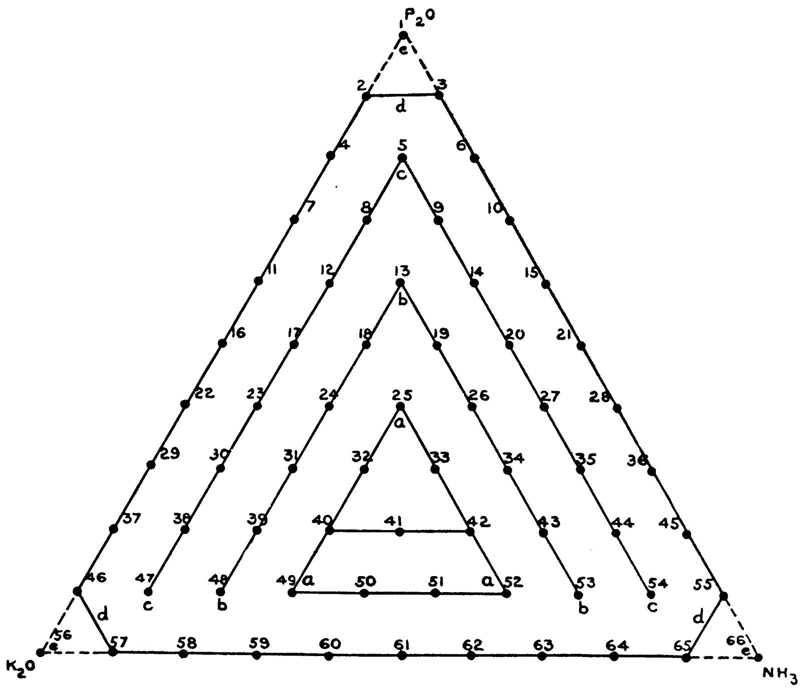

FIG. 9.—The arrangement of the culture solutions in groups: *a*, *b*, *c*, three fertilizer element groups; *d*, two fertilizer element group; *e*, one fertilizer element group.

by the individual cultures have already been pointed out, and these general impressions gleaned from the individual results can be better shown by a method of grouping. This method of grouping should be particularly valuable in showing the general tendencies, either in regard to plant growth or concentration differences connected therewith. Such a system of grouping, comparing different regions of the diagram, can be made in various ways.

The grouping shown in fig. 9 was made because by so doing the area of the greatest growth, which was always somewhat below the center of the triangle, will fall in these experiments into the central group. This central grouping is shown in the diagram as group *a*, the connecting lines showing the cultures included in this group. Group *b* takes in the cultures lying immediately outside of this central group. The same applies to group *c*, which is, however, farther removed from the central group. It will be noticed that these three groups contain the three fertilizer constituents. The next or fourth group contains all of the cultures in which two fertilizer elements occur, as shown by the lines. The fifth and last group consists of the sum of the single fertilizer elements. In handling the data, therefore, the average green weight in grams for each of these groups was determined, together with the average final concentration in the respective fertilizer constituents, or their combinations. In table I the average green weight and combined concentration of P_2O_5, NH_3, and K_2O is shown.

TABLE I

AVERAGE GREEN WEIGHT AND CONCENTRATION OF $P_2O_5+NH_3+K_2O$ OBTAINED IN DIFFERENT REGIONS OF THE TRIANGULAR DIAGRAM FIG. 9

(ppm. = parts per million)

Culture solutions	Number of cultures included	Average green weight in grams	Relative green weight	Average P_2O_5, NH_3, and K_2O		
				Original ppm.	Final ppm.	Percentage removed
Three fertilizer elements, group *a*	10	4.645	100	80	29.0	63.8
Three fertilizer elements, group *b*	11	4.120	88	80	35.0	56.3
Three fertilizer elements, group *c*	15	3.507	75	80	44.1	44.9
Two fertilizer elements	27	2.155	46	80	54.1	36.1
One fertilizer element	3	1.502	34	80	68.8	14.0
Three fertilizer elements, groups combined	36	4.010	..	80	37.0	53.8
All cultures combined	66	3.137	..	80	45.4	43.2

In the third column the relative green weight for the different groups is given, taking the results in group *a* as 100. From this it will be seen that the growth in group *b* is 88 per cent of that in group *a*, group *c* is 75 per cent; in the two fertilizer element group it is 46 per cent, and with the single fertilizer element the average result is only

34 per cent. The concentrations in these various groups are shown in the fifth column; the original concentration in all these cases was 80 parts per million of the combined constituents. In the first group, which was the one giving the greatest growth, the concentration was decreased from 80 to 29 parts per million, although, as has already been shown, the ratio of the three constituents in the solutions of this group suffered less change than did the ratio in the other groups. This can be seen from the diagram in fig. 5. In the second group the concentration was reduced to 35 parts per million; in the third group of the three fertilizer constituents to 44 parts; in the group of two fertilizer constituents it was reduced to 54 parts per million; and in the group containing one fertilizer constituent it was reduced to only 69 parts per million as an average.

For the sake of comparison, a group containing all cultures having the three fertilizer elements, as well as the combination of the entire group of sixty-six cultures, is given. The table also very clearly shows the markedly different effects produced on the green weight by a single fertilizer element and when these are used in combinations of two and of three, the results being approximately 1.5, 2.2, and 4.0, respectively, the higher group within the latter being as high as 4.6.

It will also be of interest to consider this same grouping in respect to the concentrations of the fertilizer elements individually. The number of cultures included in the group where two fertilizer elements were used was necessarily reduced, inasmuch as the ingredient in question did not occur in nine of the solutions, and the group of single fertilizer elements becomes, of course, reduced to only one culture, and for this reason has very little comparative value, although the result is usually decided enough to allow no doubt as to the general effect.

In the fifth column of table II is given the concentration of P_2O_5 found after growth, the original concentration being given in the fourth column for comparison. In the last column is shown the percentage decrease in the concentration of P_2O_5. This percentage decrease was in the first group 50 per cent, in the second group 37 per cent, and in the third group 27 per cent. In the combination of two fertilizer elements, one of which was phosphate, it

was 17.5 per cent, and in the phosphate by itself it was only 10 per cent. The actual decrease in concentration appears from the figures, however, to be strikingly uniform, varying in the different groups from 7 to 9 parts per million. This relationship of final to original concentration, however, is not properly shown by this form of grouping

TABLE II

AVERAGE GREEN WEIGHT AND CONCENTRATION OF P_2O_5 OBTAINED IN DIFFERENT REGIONS OF THE TRIANGULAR DIAGRAM FIG. 9

CULTURE SOLUTIONS	NUMBER OF CULTURES INCLUDED	AVERAGE GREEN WEIGHT IN GRAMS	RELATIVE GREEN WEIGHT	AVERAGE P_2O_5		
				Original ppm.	Final ppm.	Percentage removed
Three fertilizer elements, group *a*	10	4.645	100	16.0	8.0	49.7
Three fertilizer elements, group *b*	11	4.120	88	26.0	16.3	37.3
Three fertilizer elements, group *c*	15	3.507	75	34.0	24.9	26.9
Two fertilizer elements	18	1.531	33	40.0	33.0	17.5
One fertilizer element	1	0.648	14	80.0	71.8	10.3
Three fertilizer elements, groups combined	36	4.010	..	26.6	17.6	33.8

and can be much more satisfactorily handled, as will be done later, by grouping only such phosphate solutions as have the same original concentration. In the present table the original concentration is really an average of points varying, for instance, in the third group from 8 to 64 parts per million in phosphate content, giving the average concentration of 34 parts per million shown in the table.

TABLE III

AVERAGE GREEN WEIGHT AND CONCENTRATION OF NH_3 OBTAINED IN DIFFERENT REGIONS OF THE TRIANGULAR DIAGRAM FIG. 9

CULTURE SOLUTIONS	NUMBER OF CULTURE SOLUTIONS	AVERAGE GREEN WEIGHT IN GRAMS	RELATIVE GREEN WEIGHT	AVERAGE NH_3		
				Original ppm.	Final ppm.	Percentage removed
Three fertilizer elements. group *a*	10	4.645	100	32.0	9.8	69.4
Three fertilizer elements, group *b*	11	4.120	88	26.9	7.9	70.4
Three fertilizer elements, group *c*	15	3.507	75	22.5	5.8	59.9
Two fertilizer elements	18	2.721	58	40.0	22.8	43.1
One fertilizer element	1	2.752	34	80.0	70.2	12.2
Three fertilizer elements, groups combined	36	4.010	..	26.6	8.9	66.5

In table III is given the same character of results as have been given in the preceding section for phosphate, except that the concentration of nitrates is considered.

In the groups of two and single fertilizer elements, only those cultures containing nitrate are considered. In the three groups comprising the three fertilizer ingredients, the percentage decrease in nitrate is seen to be respectively 69, 70, and 60; in the two fertilizer element group it is 43; and in the single element, 12.

In table IV are given the results for the potash concentrations, similarly arranged and grouped as in the preceding tables.

TABLE IV

AVERAGE GREEN WEIGHT AND CONCENTRATION OF K_2O OBTAINED IN DIFFERENT REGIONS OF THE TRIANGULAR DIAGRAM FIG. 9

CULTURE SOLUTIONS	NUMBER OF CULTURES INCLUDED	AVERAGE GREEN WEIGHT IN GRAMS	RELATIVE GREEN WEIGHT	AVERAGE K_2O		
				Original ppm.	Final ppm.	Percentage removed
Three fertilizer elements, group *a*..	10	4.645	100	32.0	10.8	66.2
Three fertilizer elements, group *b*..	11	4.120	88	26.9	11.3	58.0
Three fertilizer elements, group *c*..	15	3.507	75	22.9	9.6	58.3
Two fertilizer elements..........	18	2.212	48	40.0	26.1	34.8
One fertilizer element............	1	1.106	24	80.0	44.3	44.6
Three fertilizer elements, groups combined..................	36	4.010	..	26.6	10.4	60.9

The percentage decrease in the cultures is for the three fertilizer elements in the three groups 66, 58, and 58, respectively; for the two fertilizer elements, 35; and for the single fertilizer element, 45.

Influence of different amounts of P_2O_5, NH_3, and K_2O, varying, respectively, from 8 to 80 parts per million

In the consideration of the results up to this point, no special discussion has been made in regard to the actual amounts of the individual fertilizer ingredients which were in the cultures. By reference to the original description of the diagram fig. 1 it will be seen, for instance, that there was a series of cultures in the line from number 46 to number 55 which contained 10 per cent of phosphate in the fertilizer mixture, or 8 parts per million of the culture solutions. Similarly, the line 37 to 45 contained 16 ppm.; the line 29 to 36, 24 ppm.;

and so on to the apex of the triangle. In the same manner, the line of cultures from 3 to 57 contained 8 ppm. NH_3 as nitrate; the line 6 to 58, 16 ppm.; and so on up to 80 ppm. NH_3 in culture 66. The line of cultures from 2 to 65 contained 8 ppm. K_2O; line 4 to 64, 16 ppm.; and so on up to 80 ppm. K_2O in culture 56.

It will be interesting to see what effect these different amounts of each fertilizer ingredient have had on the growth of the plant, and the removal of this constituent during the period of growth. In such

TABLE V

SHOWING THE RESULTS OBTAINED FOR P_2O_5 IN THE CULTURES CONTAINING DIFFERENT AMOUNTS OF P_2O_5 (PPM. = PARTS PER MILLION)

Percentage of P_2O_5 in fertilizer mixture	Parts per million P_2O_5 in solution	Average content of P_2O_5 in final solution, ppm.	Average decrease of P_2O_5		Average green weight	P_2O_5 decrease per unit of green weight
			ppm.	percentage		
100	80	71.8	8.2	10.3	0.648	12.7
90	72	61.5	10.5	14.6	1.226	8.6
80	64	54.1	9.9	15.5	1.983	5.0
70	56	46.2	9.8	17.5	2.580	3.7
60	48	37.8	10.2	21.3	3.045	3.3
50	40	29.2	10.8	27.0	3.340	3.2
40	32	22.2	9.8	30.6	3.295	3.0
30	24	16.2	7.8	32.6	3.308	2.3
20	16	9.2	6.8	42.5	3.558	2.9
10	8	2.9	5.1	63.8	3.640	1.4
0	0	....	....	....	3.135	...

consideration it must always be borne in mind that there were present at the same time varying quantities of the other fertilizer constituents. For the purpose of such a comparison, the growth and concentration in the cultures along any of these lines has been studied and is presented in this section. With this in view all the results obtained in the various cultures in any one line were added together, and as the number of cultures naturally differ from line to line, the average was determined in each case for comparison, although this average is not intended to mean that all the cultures along this line were similar, for it has already been shown that in general the points in the middle were higher than those at the end of any one of these lines.

PHOSPHATE.—In table V are given the results obtained for P_2O_5 in the cultures containing different amounts of P_2O_5, varying from 80 to 8 ppm., as shown in the second column of the table.

The first column gives the percentage composition of the fertilizer mixture so far as the P_2O_5 content was concerned. In the third column is given the average concentration for the eight three-day periods in the experiment, and in the fourth column, the difference between this and the original concentration, thus giving the average decrease, which is expressed as a percentage in the fifth column on the basis of the original concentration. The sixth column gives the average green weight obtained along any of these concentration lines, and the next column gives the decrease in P_2O_5 calculated to the unit basis of one gram production of green plant. In other words, the decrease recorded in the fourth column has been divided by the green weight corresponding thereto in the sixth column. This gives, as it were, the rate of decrease in parts per million of the solution for each gram of green weight produced; but if it is desired to have the result expressed in terms of milligrams of P_2O_5 removed by each gram of green weight produced, the figures in the column must be multiplied by 2, since 250 cc. of solution were presented to the plants eight times; i.e., a total of 2000 cc. It will be noticed that the green weight steadily increased as the phosphate content decreased. It must be borne in mind, of course, that when the phosphate content decreases, there is a corresponding increase in the average content of both potash and nitrogen. In the last figure for the green weight, however, namely that in which the phosphate content became zero in the culture solutions, there is again a marked drop, although the potash and nitrogen content in these was higher than in any of the solutions above this in the table. It follows accordingly that a very distinct part was played by the phosphate in producing growth, although its maximum efficiency seems to be reached in these experiments in rather low concentration. Attention might also be called here to the fact that the concentrations of phosphate in soil solutions are always low, and relatively much lower than any of the other constituents here considered. The plant in its natural environment, therefore, has adapted itself to the occurrence of this constituent in weak solutions.

The increase in green weight shown by the table to be from 3.1 to 3.6 grams corresponded to an increase from 0 to 8 ppm. in the original P_2O_5 content. The further increase to 16 ppm. P_2O_5 has

produced no further increase in plant growth, and above this a decrease has even followed, which may be in part due to some direct influence of the phosphate solution, the carrier here used being calcium acid phosphate, and may also be due in part to the fact that the quantities of potash and nitrate are being decreased as the phosphate increases. This relation, however, has been discussed in a much more thorough manner where the ratios of these substances are considered, and the change in concentration which the solutions undergo, as shown in the diagram of ratio change in fig. 5, makes this matter very clear. It is there shown that the region of better growth is found in the lower part of the diagram, and this is also the region of most absorption and least change in the ratio. All these regions it will be noticed comprise solutions which contain low amounts of P_2O_5.

The average concentration of phosphate in the culture solution after growth, as shown in the third column, continually decreases. The actual decrease in the next column, however, shows that the greatest decrease in concentration of this element does not go with the greatest growth, but is found in the 40 ppm. phosphate solutions, increasing steadily from the 8 ppm. solution to this point, and then becoming practically constant up to the point where the other fertilizer elements entirely disappear and only phosphate remains, when it again drops slightly, the conditions for growth also being much poorer through this total absence of potash and nitrogen. The decrease when expressed as a percentage of the amount of material originally present shows that the removal is the more complete as the original concentration is lower. The last column in this section of the table, giving the decrease per unit of green weight, shows that equal weights of green plants cause quite unequal decreases in concentration, this change being least in the weaker solutions and greatest in the higher, thus indicating that more was removed by the plant in the higher concentrations than it could economically utilize under the conditions; i.e., the plant absorbed the material because it was there, although it apparently had already all that it could utilize economically. This is a fact consistent with field observations and ash analysis, where it is frequently noticed that the actual amount of mineral constituents is larger in the poorer plants.

NITRATE.—Table VI gives the results obtained for nitrate in the cultures containing different amounts of nitrate, similarly arranged and computed as in the phosphate table just discussed.

TABLE VI

SHOWING THE RESULTS OBTAINED FOR NH_3 IN THE CULTURES CONTAINING DIFFERENT AMOUNTS OF NH_3 (PPM. = PARTS PER MILLION)

PERCENTAGE OF NH_3 IN FERTILIZER MIXTURE	PARTS PER MILLION OF NH_3 IN SOLUTION	AVERAGE CONTENT OF NH_3 IN FINAL SOLUTION, PPM.	AVERAGE DECREASE OF NH_3		AVERAGE GREEN WEIGHT	NH_3 DECREASE PER UNIT OF GREEN WEIGHT
			ppm.	percentage		
100	80	70.2	9.8	12.3	2.752	3.6
90	72	54.2	17.8	24.7	2.699	6.6
80	64	42.0	22.0	34.3	3.046	7.2
70	56	32.8	23.2	41.4	3.169	7.3
60	48	24.1	23.9	49.8	3.492	6.8
50	40	18.3	21.7	54.2	3.685	5.9
40	32	12.0	20.0	62.5	4.166	4.8
30	24	6.1	17.9	74.6	3.852	4.6
20	16	0.5	15.5	96.3	3.893	4.0
10	8	0.2	7.8	97.5	3.156	2.5
0	0	....	....	....	0.995	...

The green weight column differs from the one discussed in the phosphate table in that the highest result occurs with the 32 parts per million NH_3 line, the numbers descending in each direction from his higher green weight. The change from 0 to 8 ppm. NH_3 is very marked in the green weight, which increases from approximately 1.0 gram to 3.2 grams, an even greater change than was noted in the phosphate table. The average decrease in concentration was in this case greatest between the limits of 32 to 64 ppm., the increase being gradual from the 8 ppm. solution up to the 32 ppm. solution, then being practically uniform up to 64 ppm., when it again declines. The figures in the percentage decrease column are on the whole much greater than those in the corresponding column in the phosphate table, thus showing a comparatively greater decrease of the nitrogen than of the phosphate in solutions of equal content of these elements, respectively. The last column, showing the rate of decrease per unit of green weight, shows the same general tendency for these figures to decrease with the decline in the original content of nitrogen in the solutions, as was noticed in the phosphate table, except that

the first two results, namely in the 80 and 72 ppm. solutions, are in this case lower than the following three or four entries, while in the phosphate table these two were the highest in the column. The conditions for growth with the phosphate in these two solutions, or groups of solutions, were very poor as compared with the rest of the results, while with the nitrate this difference in growth was not so apparent.

POTASSIUM.—In table VII are given the results obtained for potassium, the table being arranged and the results computed in the same manner as with those in preceding tables.

TABLE VII

SHOWING THE RESULTS OBTAINED FOR K_2O IN THE CULTURES CONTAINING DIFFERENT AMOUNTS OF K_2O (PPM. = PARTS PER MILLION)

Percentage of K_2O in fertilizer mixture	Parts per million of K_2O in solution	Average content of K_2O in final solution (ppm.)	Average decrease of K_2O		Average green weight	K_2O decrease per unit of green weight
			ppm.	percentage		
100	80	64.4	15.6	19.5	1.106	14.1
90	72	54.1	17.9	24.8	1.922	9.3
80	64	40.6	23.4	36.5	2.661	8.8
70	56	36.9	19.1	34.1	2.890	6.6
60	48	28.9	19.1	39.8	3.222	5.9
50	40	20.6	19.4	48.5	3.600	5.4
40	32	11.5	20.5	64.0	3.929	5.2
30	24	6.2	17.8	74.2	3.877	4.6
20	16	3.9	12.1	75.6	3.538	3.4
10	8	3.1	4.9	61.3	3.172	1.6
0	0	....	....	....	1.978	...

The green weight column is in its general tendency very similar to that observed in the nitrate table, namely that the 32 ppm. cultures give the highest green weight. The figures representing the average decreases and percentage decreases, on the other hand, correspond in trend more closely with those shown in the phosphate table, this similarity being especially shown in the last column giving the rate of decrease per unit of green weight, in which a regularly descending column of figures is seen. In the case of the green weight, the change from the 8 ppm. solutions to those containing no potash whatever was, as in the other cases, very marked, and this drop in green weight was also distinctly noticeable in the solution where potash alone was present. The results given in the three columns in the tables showing

the rate of decrease per unit of green weight are consistent in showing that relatively more was absorbed when more was present, although the plant does not seem to have been able to utilize this increase economically in its growth. This tendency is especially marked in the case of phosphate and potash, although it is also shown to an appreciable extent in the case of nitrogen.

As will appear in future publications, this general method of experimentation was used for the purpose of studying the effect of individual soil contituents and other organic compounds by using them in uniform concentration in all the cultures of a triangle. In these studies it was necessary to grow a control set without the constituent to be studied, so that the foregoing experiment was in this manner repeated a number of times, and the general results thus obtained were in harmony with those here recorded.

Summary

In this study the growth relationships and concentration differences were observed between solution cultures in which the phosphate, nitrate, and potash varied from single constituents to mixtures of two and three in all possible ratios in 10 per cent stages.

The better growth occurred when all these nutrient elements were present, and was best in those mixtures which contained between 10 and 30 per cent phosphate, between 30 and 60 per cent nitrate, and between 30 and 60 per cent potash. The growth in the solutions containing all three constituents was much greater than in solutions containing two constituents, the solutions containing the single constitutent giving the least growth.

The concentration differences noticed in the solutions were also very striking, the greater reduction in concentration occurring where the greatest growth occurred.

The change in the ratios of the solutions and the ratios of the materials that were removed from the solutions showed that where the greatest growth occurred, as above outlined, the solutions suffered the least change in ratio, although the greatest change in concentration occurred.

The more the ratios in these solutions differed from the ratios in which the greatest growth occurred, the more were the solutions altered

in the course of the experiment, the tendency in all cases seeming to be for the plant to remove from any and all of these solutions the material in the ratio which normally existed where greatest growth occurred. This did not actually occur in all cases, owing to the unbalanced condition of some of the solutions.

The results show that the higher the amount of any one constituent present in the solution, the more does the culture growing in that solution take up of this constituent, although it does not seem able to use this additional amount economically.

In the very early periods the ratio of phosphate absorption is low and the potash absorption high, although in final growth the greater response is obtained with nitrate, indicating relatively low phosphate requirement and high potash requirement of the seedling plant.

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